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# Cultivating soil life: a diluted compost extract solution stimulated arbuscular mycorrhizal symbiosis in *Myrcianthes cisplatensis* seedlings

Cultivando a vida do solo: uma solução diluída de extrato de composto estimulou a simbiose micorrízica arbuscular em mudas de *Myrcianthes cisplatensis*

Cultivando la vida del suelo: una solución diluída de extracto de compost estimuló la simbiosis micorrícico arbuscular en plantines de *Myrcianthes cisplatensis*

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## Abstract

Inspired by the living soil theoretical framework and agroecological principles, we designed an experiment to study the effect of compost extract on arbuscular mycorrhizal symbiosis (AMS) with the argentinean native tree *Myrcianthes cisplatensis*. *M. cisplatensis* seedlings were transplanted in 1 lt pots with 10% of soil enriched with indigenous AMF populations and some plants were irrigated with 50 mL of 1:500,000 compost extract dilution three days after the transplant. Thus, the experiment had four treatments: AMF; no AMF (autoclaved soil); AMF+compost extract; AMF+compost extract filtered through a nanopore filter (0.45 µm pore diameter). Plants were grown for 150 days. We found that the dilution of the compost extract stimulated AMS independently of the presence of microorganisms in the extract, strongly suggesting that the stimulation was due to the presence of soluble biomolecules synthesized by the metabolism of the compost. Our results contribute to the generation of knowledge regarding the underlying mechanisms of biological amendments, in this case a compost extract, on the stimulation of AMS with native tree seedlings.
**Keywords:** Living soil, soil metabolism, trap culture, biological amendments.

## Resumo

Inspirados no referencial teórico do solo vivo e princípios agroecológicos, projetamos um experimento para estudar o efeito do extrato de composto na simbiose micorrízica arbuscular (SMA) com a árvore nativa argentina *Myrcianthes cisplatensis*. As mudas de M. cisplatensis foram transplantadas para vasos de 1 lt com 10% de solo enriquecido com populações indígenas de FMA e algumas plantas foram irrigadas com 50 mL de diluição de extrato de composto 1:500.000 três dias após o transplante. Assim, o experimento teve quatro tratamentos: FMA; sem FMA (solo autoclavado); FMA+extrato de composto; FMA+extrato de composto filtrado através de um filtro nanopore (diâmetro do poro 0,45 µm). As plantas foram cultivadas por 150 dias**.** Descobrimos que uma diluição de 1:500.000 do extrato de composto estimulou a SMA independentemente da presença de microrganismos no extrato, sugerindo fortemente que a estimulação foi devido à presença de biomoléculas solúveis sintetizadas pelo metabolismo do composto. Nossos resultados contribuem para a geração de conhecimento sobre os mecanismos subjacentes de emendas biológicas, neste caso um extrato de composto, na estimulação da SMA com mudas de árvores nativas.
**Palavras-chave:** Solo vivo, metabolismo do solo, cultura armadilha, emendas biológicas.

## Resumen

Inspiradas en el marco teórico del suelo vivo y los principios agroecológicos, diseñamos un experimento para estudiar el efecto del extracto de compost sobre la simbiosis micorrícica arbuscular (SMA) con el árbol nativo argentino *Myrcianthes cisplatensis*. Las plántulas de *M. cisplatensis* se trasplantaron en macetas de 1 lt con 10% de suelo enriquecido con poblaciones autóctonas de HMA y algunas plantas se regaron con 50 mL de una dilución 1:500.000 de extracto de compost tres días después del trasplante. Así, el experimento tuvo cuatro tratamientos: HMA; sin HMA (suelo esterilizado en autoclave); HMA+extracto de compost; HMA+extracto de compost filtrado a través de un filtro nanopore (diámetro de poro 0,45 µm). Las plantas se cultivaron durante 150 días. Encontramos que una dilución 1:500,000 del extracto de compost estimuló la SMA independientemente de la presencia de microorganismos en el extracto, lo que sugiere fuertemente que la estimulación se debió a la presencia de biomoléculas solubles sintetizadas por el metabolismo del compost. Nuestros resultados contribuyen a la generación de conocimiento sobre los mecanismos subyacentes de las enmiendas biológicas, en este caso un extracto de compost, sobre la estimulación de SMA con plántulas de árboles nativos.
**Palabras-clave:** Suelo vivo, metabolismo de suelo, cultivo trampa, emiendas biológicas.

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Climate change scenario evidences the urgent necessity for a change towards ways of production that understand the ecological and social processes underlying it and that takes into consideration the impact on common goods for future generations. Agroecology is a paradigm that designs and develops actions and practices relying on those conceptions (FAO, 2018; Wezel *et al*., 2020). The concepts used in agroecology are dynamic, they evolve as more actors get involved in the search for solutions that contribute to sustainability (Wezel *et al*., 2020).

The living soil theory nourishes agroecology (Balfour, 1945). This theory, proposed by Eva Balfour in 1945, and developed subsequently by others authors (Primavesi, Molina, 1984; Sanchez *et al.*, 2012), considers soil as a living organism with a metabolism and the capacity for auto perpetuation. Soil metabolism is composed of complex and diverse interactions between the organisms that live within the soil (i.e.: bacteria, fungi, mesofauna, fauna and plants). Organisms may interact in a food chain, in intimate contact with physical association regardless beneficial effect (symbiosis), in physical association were one organisms benefits and the other/-s do not (commensalism) or compete with each other when they need a resource that is scarce (competition) (Margulis, 1990). All types of interactions can co-exist in an ecosystem, they not exclude each other.

Organisms have co-evolved in interaction and thus have developed strategies of communication. An example of this co-evolution is the Arbuscular Mycorrhizal Symbiosis (AMS), a symbiotic relation between Arbuscular Mycorrhizal Fungi (AMF) and terrestrial plants (AMS have been reported in more than 90% of terrestrial plants species, including mosses) (Brundett, Tedersoo, 2018; Trivedi *et al.*, 2020). Several authors consider AMF as living fossils since there is evidence that they played a significant role during the establishment of terrestrial plants (Parniske, 2008; Trivedi *et al.*, 2020). The symbiosis allows the exchange between organisms of minerals from rocks (AMF to plants) and carbon from photosynthesis (plants to AMF). The symbiotic relation also promotes plant health by inducing systemic induced resistance (SIR) (Pieterse *et al.*, 2014).

Arbuscular mycorrhizal symbiosis can be considered as a dynamic link between plants and soil metabolism. Arbuscular mycorrhizal fungi have a wide plant host range and there is evidence that supports the idea that different plants (i.e. species, genre and family) can interconnect by the common mycorrhizal network (CMN) (Giovannetti; Avio; Sbrana, 2015). Moreover, it has been demonstrated that mycelium from individuals of the same AMF species isolated from the same population can merge through the anastomosis of their hyphae whereas hyphae from the same AMS species but isolated from a different population cannot (Giovannetti; Avio; Sbrana, 2015). Thus it seems logical that agronomic practices that seek for stimulation of AMS should consider the interaction with the pre-existing AMF community.

One strategy is to cultivate the AMF community that lives in the agroecosystem *ex situ* and then design its re-introduction (i.e. transplantation of seedlings in symbiosis with the indigenous AMF community or population), known as on farm multiplication. This is a low cost technique and reproduces native AMF populations. Some of its drawbacks are that it demands a lot of space and that the application of the AMF populations could be difficult for big land extensions (Kumar and Sakena, 2017).

A complementary approach to stimulate soil life and particularly AMS is the use of biological amendments. Application of biological amendments (i.e. compost, supermagro, bokashi) could stimulate the symbiosis via the biomolecules synthesized as a consequence of the metabolism created (Crespo and Frank, 2022). Our group have recently showed that an application of supermagro (SPM) in *Triticum aestivum* seeds, in a proportion of 150 ml of SPM per 40 kg of seeds, improved AMS and Zinc concentration in plant tissues and grains (de la Torre *et al.*, 2022). These results triggered the questions: Which is/are the mechanism/-s underlying AMS stimulation? Is the presence of microorganisms in the SPM that stimulated AMS or is the presence of soluble biomolecules synthesized by the metabolism created by the microorganisms in the biological amendment?

As a first approach to answer these questions we designed an experiment to study the effect of a compost extract dilution on AMS. The aims of the experiment were to evaluate the effect of a compost extract dilution, in an order of $1 \times 10^{-6}$, on AMS and to

elucidate if the possible effect depended or not on the presence of microorganisms in the compost extract dilution.

We selected as a plant model the argentinean native tree *Myrcianthes cisplatensis* and a soil enriched with AMF populations isolated from a *M. cisplatensis* forest as the source for the symbiosis establishment. *Myrcianthes cisplatensis* is a tree that belongs to the Myrtaceae family and inhabits the Chaco Province, the Espinal and the transition with the Paranaense Province (Cabrera and Willink, 1980). It grows on river or streams banks and in forests and gallery jungles. It can be used for alignment trees, forest curtains, combined in agroecosystems, among other uses (Eynard; Calviño; Ashworth, 2017). Its potential is due to characteristics related to the quality of the wood, its rusticity and speed to grow. In addition to being an ornamental plant due to its attractive bark and foliage, it is melliferous, medicinal and its fruits are edible (Eynard; Calviño; Ashworth, 2017).

Experimental set-up

i) Development of a soil enriched with AMF associated with *M. cisplatensis in situ*

A set of ten trap cultures (TC) were prepared with soil obtained from a grass patch growing beside a natural population of *M. cisplatensis* (32°29'16.1" S 60°50' 57.5" O; south Santa Fe province, Argentina) in order to reproduce indigenous AMF populations. An area of 8 $m^2$ was delimited and five soil samples were randomly collected; a total of 40 lts of soil were sampled. *Myrcianthes cisplatensis* seedlings were also sampled. Seedlings and soil were transported to the greenhouse, mixed with autoclaved sand (1:1) and the mixture was used to fill ten 4 lts pots. *Sorgum bicolor* seeds (Los Prados S.A. facilitated by Instituto Nacional de Semillas - INASE, Argentina) were planted, at a proportion of 50 seeds per $m^2$. together with one seedling of *M. cisplatensis*, so that the TCs were also under the influence of *M. cisplatensis* (Gil-Cardeza *et al.*, 2018). *Sorgum bicolor* were grown under semi-controlled conditions (controlled irrigation, without control on photoperiod and temperature) for 6 months, alternated with *Triticum aestivum* plants seeded at the same proportion for the winter

period, and after that, *S. bicolor* seeds were re-planted and grown for 4 months until evaluation.

At this time, 3 sorghum plants were harvested from six TC, roots were separated, cleaned and stained for the determination of AMS structures (see Mycorrhizal determination sub-section at MandM). The TC were selected randomly from the ten original TC. The percentages of mycorrhizal intensity (I%) obtained were: TC1 = 21%, TC2 = 28%, TC3 = 30%, TC4 = 14%, TC5 = 14% and TC6 = 6%.

*Myrcianthes Cisplatensis* seedling obtention - Approximately, 200 *M. cisplatensis* seeds were collected from the natural population site (32°29'16.1" S 60°50' 57.5" O) and were germinated under controlled conditions in plastic trays with blotting paper (25-27 °C, constant humidity with reverse osmosis water, photoperiod of 12 h) (Eynard; Calviño; Ashworth, 2017). Seeds germinated within 6 - 10 days; individual seedlings were transplanted to pots of 100 mL filled with perlite, peat and compost in a proportion of 5:3:2, respectively. *M. cisplatensis* were grown under semi-controlled conditions (controlled irrigation, without control on photoperiod and temperature) for 11 months; the seedlings were watered to field capacity regularly.

ii) Compost elaboration

The compost used for the experiment was produced at the Vivero Forestal Agroecológico from the Facultad de Ciencias Agrarias, Universidad Nacional de Rosario, Argentina. Organic wastes, predominantly pumpkin and potato peel, lettuce, onion and tomato, from the university cafeteria were mixed with *Populus sp.* and *Ligustrum lucidum*dry leaves at a 1:1 proportion and were disposed of as a compost pile over the soil. Organic wastes and leaves were weekly incorporated into the pile, at the same 1:1 proportion. The compost was watered frequently and the pile was periodically turned over during 4 months in order to avoid anoxic conditions. Humidity was checked weekly with the hand squeeze test (Román; Martínez; Pantoja, 2013). Organoleptic properties (color, structure, odor, moisture and color of the suspension) and the maturity of the compost were assessed by La Enmienda (Table 1). The maturity of the compost was determined by the quantification of the trophic microbiologic network (Inghman

and Slaughter, 2004). Briefly, compost samples are dissolved 1:5 in water and 25 fields of water drops are observed under a light microscope. The different metabolic links are quantified following the protocol developed by Dra. Elaine Ingham (Inghman and Slaughter, 2004). The trophic microbiologic network analysis established that the compost was almost mature. Compost C:N relation was 11% (HSE soluciones, Argentina, with S.A.M.L.A. method) .

**Table 1.** Organoleptic properties and trophic biological network of compost. One hundred grams of compost was sampled and send for further analysis to La Enmienda, were the analysis are made following the protocol developed by Dra. Elaine Inghman (Inghman and Slaughter, 2004). Data is expressed as media and standard error of 25 field observations.

| Organoleptic properties | |
|---|---|
| Color | Chocolate brown |
| Structure | Micro and macro aggregates |
| Odor | Soft |
| Moisture | Medium |
| Color of suspension | Diluted coffee |
| **Trophic biological network** | |
| Link | Value |
| Bacteria | (950 ± 441) $\mu g*g^{-1}$ |
| Actinobacteria | 0 |
| Fungi | (145 ± 112) $\mu g*g^{-1}$ |
| Hypha diameter | (3.90 ± 1.5) µm |
| Ratio F : B | 0.15 |
| Total protozoa | (73,368 ± 51,879) $\mu g*g^{-1}$ |
| Amoebas | (73,368 ± 51,879) $\mu g*g^{-1}$ |
| Flagellated | 0 |
| Ciliates | 0 |
| Oomycetes | (9.70 ± 21.70 ) $\mu g*g^{-1}$ |
| Plant pathogenic nematodes | 0 |
| Bacteriophage nematodes | 0 |
| Fungivorous nematodes | 0 |
| Nematode predators | 0 |

**Source**: La Enmienda, 2022.

iii) Compost extract preparation

A compost extract was prepared in order to evaluate the effect of compost on AMF symbiosis with *M. cisplatensis* seedlings. The compost extract was prepared from a

compound compost sample by an incubation of 10 minutes of 500 gr of compost (inside a mesh, 400-500 µm pore diameter, La Enmienda) in 20 lts of reverse osmosis water. Mineral (Table 2) and potentially toxic elements (Table 3) in compost extract were quantified by ICP-MS (Perkin-Elmer, CCT Rosario) together with total Nitrogen determination (HSE soluciones, with Kjeldahl method).

For the evaluation of the effect of compost extract on AMF symbiosis, a 1:500,000 dilution was made with reverse osmosis water. In order to distinguish between the effect of microorganisms and the effect of biologically active molecules, the dilutions were filtered with a nanopore filter (0.45 µm pore diameter). Dilution selection was made considering previous results of the research group (de la Torre *et al.*, 2022).

**Table 2:** Element composition of Compost Extract (CE). Compost Extract element composition was quantified by ICP-MS. Total amount (mg) of each element present in the 50 ml of the 1:500,000 dilution used at the experiment is expressed together with the concentration and total amount of each element present in the low P Hoagland solution used as the nutritive solution during the experiment.

| Element | CE (mg $*L^{-1}$) | Total mg in 50 ml CEdil | Low P Hoagland | Total mg in 100 ml Hoagland |
|---|---|---|---|---|
| B | 768,867 | 0.0769 | 0.25 | 0.025 |
| Ca | 4,939,195 | 0.4939 | 140 | 14 |
| Co | 707 | 0.0001 | 0 | 0 |
| Cu | 25,191 | 0.0025 | 0.0381 | 0.00381 |
| Fe | 280,697 | 0.0281 | 2.9 | 0.29 |
| K | 16,858,715 | 1.6859 | 235 | 23.5 |
| Mg | 1,436,853 | 0.1437 | 24 | 2.4 |
| Mn | 5,202 | 0.0005 | 0.19 | 0.019 |
| N | 1,400 | 0.0001 | 42,422 | 4242.2 |
| Na | 15,287,362 | 1.5287 | 0.025 | 0.0025 |
| P | 4,050,464 | 0.4050 | 6 | 0.624 |
| Se | 1,202 | 0.0001 | 0 | 0 |
| Zn | 10,889 | 0.0011 | 0.14 | 0.014 |

Source: Fernández, 2022.

**Table 3:** Potentially Toxic Element composition of Compost Extract (CE). Compost Extract Potentially Toxic Element (PTE) composition was quantified by ICP-MS. Total amount (mg) of each PTE present in the 50 ml of the 1:500,000 dilution used at the experiment is expressed.

| Potentially toxic element | CE (mg *$L^{-1}$) | Total mg in 50 ml CEdil |
|---|---|---|
| Al | 112,906 | 0.0113 |
| As | 1,129 | 0.000113 |
| Cr | 6,469 | 0.000647 |
| Hg | 856 | 0.0000856 |
| Ni | 1,581 | 0.000158 |
| Pb | 944 | 0.000094 |

**Source**: Fernández, 2022.

iv) Experimental design

Eleven-month *M. cisplatensis* seedlings were transplanted to 1 lt pots previously filled with a mixture of autoclaved (121 °C, 30 minutes) sand : perlite (4:1 proportion) up to 50% of its volume. A fine layer of the TC mixture (100 mL) was added over the autoclaved substrate. Then, pots were filled to complete its volume with the same mixture of substrate. Previous to the transplant, *M. cisplatensis* seedlings height was measured so that each treatment contained a representative group of seedlings. The treatments were: Inoculation with 100 mL of the TC (AMF); AMF + 1:500,000 filtrate compost extract (AMF+$CE_F$ dil); AMF + 1:500,000 compost extract (AMF+CE dil) and AMF with autoclaved TC (no AMF). Each treatment consisted of 5 replicates. The TC used for the AMF treatments was a mixture of TC1, TC2 and TC3; a mixture of TC4, TC5 and TC6 was autoclaved and used for the no AMF control. The compost extract treatment consisted in one irrigation with 50 mL of each dilution, 3 days after the transplant. Plants were grown for 6 months. They were regularly irrigated with 100 mL of low phosphorous Hoagland solution (Gil-Cardeza *et al.*, 2021).

v) Determinations and analysis

*Myrcianthes cisplatensis* growth-related - Seedlings height (from the base of the pot to the apical meristem) was measured at 30, 60, 90 and 150 days. At the end of the experiment, plants were harvested, shoots were separated from the roots and fresh

weight was measured. Then, the tissues were dried at 80 °C for 96 h and dry weight was determined. Both determinations were made with an analytical balance (0.001 gr).

Arbuscular mycorrhizal symbiosis - A representative portion of dry roots were placed in Falcon tubes and re-hydrated for 48 h in deionized water (Gil-Cardeza *et al.*, 2021). The roots were then incubated at 70 °C in a water bath for 30 min in 50 mL KOH 10% w/V (Cicarelli, pro-analysis), KOH was discarded and fresh 50 ml were added and roots were incubated for another 30 min at 70°C. The KOH was removed and roots were rinsed thoroughly with tap water and rinsed for 5 min with HCl 1% w/V (Cicarelli, pro-analysis (36.5-38% w/w)). Roots were further stained in 25 mL of cotton blue 0.05% w/V (Biopack, suitable microscopy) containing HCl (0.05% w/V) and glycerin (50% w/V; Biopack, pro-analysis (99.5% w/w)) and incubated at 90 °C in a water bath for 20 min. The roots were finally rinsed and stored in acid glycerin before observation (Phillips and Hayman, 1970).

For observation, twenty root fragments of ~10 mm length were mounted on microscope slides and examined under a compound microscope at 20-40x magnifications. The intensity of mycorrhizal association (%I) was calculated as follows: (v + 5w + 30x + 70y + 95z)/(v+w+x+y+z), where v, w, x, y , z are the number of root fragments containing an increasing proportion (i.e. v: <1%, w: 1-10%, x: 11-50%, y: 51-90%,z: > 90%) of AMF structures (Dodd *et al*., 2001).

Statistical analysis - All analyses were conducted using INFOSTAT (Di Renzo *et al.*, 2016). Differences between averages of Intensity of the mycorrhizal association (%I) and fresh and dry weight were analyzed with One-way ANOVA ($p \leq 0.05$). Multiple comparisons between medias were made with the Tukey post test ($p \leq 0.05$). Intensity of the mycorrhizal association didn't assume the assumptions for homoscedasticity so data were transformed with arcsine $\sqrt{(x+1)}$. Height curves were analyzed with the repeated measures test ($p \leq 0.05$).

Compost extract effect on AMS and growth of *M. cisplatensis* seedlings - All *M. cisplatensis* seedlings grown in presence of viable AMF established the symbiosis (Fig. 1). The highest mycorrhizal intensity values (%I) were observed in *M. cisplatensis* roots irrigated with the 1:500,000 compost extract dilution, independently of the filtration

through the nanopore filter: 31% no filtration and 26% filtrated (Fig. 1 AMF+$CE_{dil}$ and AMF+$CE_{Fdil}$, respectively). Mycorrhizal intensity in *M. cisplatensis* control AMF roots was 6%. No AMF structures were observed in no-AMF control.

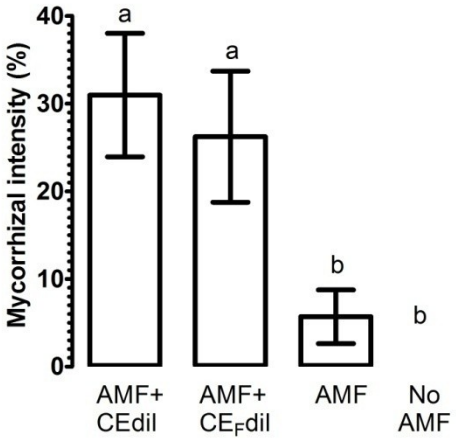


**Figure 1:** Mycorrhizal intensity (%I) in *Myrcinathes cisplatensis* roots. Eleven months old *M. cisplatensis* seedlings were transplanted to 1lt pots filled with autoclaved substrate with (AMF) or without (no AMF, autoclaved TC) arbuscular mycorrhizal fungi. Three days after the transplant pots were irrigated with 50 mL of a dilution of compost extract previously filtered or not filtered through a nanopore filter (1:500,000 dilution without filtration: AMF + $CE_{dil}$; 1:500,000 dilution filtered AMF + $CE_{Fdil}$). Data are expressed as means ± SEM (N = 4). Values with the same lower-case letters in a graph do not differ at $P \leq 0.05$ (one-way ANOVA; multiple-comparisons were made by a multiple range Tukey test).

These findings show a stimulatory effect on AMS of a 1:500,000 compost extract dilution. Among the variables that could influence the symbiosis are minerals, microorganisms and hormones, growth factors and/or other metabolic products of microbial activity developed in the metabolism of the compost (Parniske, 2008; Koskey *et al.*, 2022). The experimental design carried out, in the sense of the dilution chosen and the selection of a single application of the compost extract at the beginning of the experiment, reduced the possibility of an influence on AMS of the minerals present in the compost extract. This is in accordance with the mineral concentrations determined in the compost extract and the amounts administered by Hoagland's solution, where it was observed that the amount of minerals provided by the extract is lower than those provided by the solution (Table 2).

Regarding the microorganisms present in the compost extract, from the results obtained it can be deduced that they didn't have an effect on mycorrhization themselves since mycorrhization was promoted with the same intensity in seedlings irrigated with the compost extract 1:500,000 dilution, with or without microorganisms. This result suggests that the mycorrhization promoted by the 1:500,000 compost extract dilution could be due to the presence of hormones, growth factors or other metabolic products present in the extract as a result from the microbial activity of compost. This group of variables exert their effects at very low concentrations, normally around picomole (Ázcon-Bieto and Talón, 2008; Taiz and Zeiger, 2010).

The biological analysis of the solid compost indicated the absence of potentially pathogenic microorganisms and that the biological composition was not complete according to the parameters established by the La Enmienda laboratory (Soil Food Web School, Table 1). The biological composition was not complete since a high proportion of bacteria, with respect to the fungal biomass, was determined. This could be due to the low proportion of carbon materials used during the preparation of the compost (C-N ratio, 10:1). Therefore, it would be interesting to evaluate the effect of a dilution, within the order of 1 x $10^{-6}$, of the compost extract using a solid amendment of complete biological composition, since it is possible to assume that the stimulatory effects on mycorrhization could be even greater if the compost used had a complete biological composition (Cozzolino *et al.*, 2016).

Even though it was not the principal aim of the work, height (Fig. 2) and weight (dry and fresh) were determined on *M. cisplatensis* seedlings, so as to gather more knowledge regarding the effects of the treatments. *M. cisplatensis* seedlings that grown in presence of AMF and that were irrigated with the 1:500,000 compost extract dilution, independently of the filtration treatment, showed the highest values of height at the end of the experiment (Fig. 2, ○ and ●, (36 ± 3) and (35 ± 3) cm, respectively). No AMF control seedlings heights (Fig. 2, □, (31± 2) cm) did not differed statistically from heights of AMF controls seedlings (Fig. 2, ▪, (29 ± 2) cm). These findings are in accordance with the stimulation on AMS observed in *M. cisplatensis* roots that grown in the pots irrigated with the compost extract. Since it was not the principal aim of the

work, no control without AMF and compost extract irrigation was made, so it is not possible to conclude a cause-effect relation between AMS stimulation and the higher heights for observed in the compost extraction treatments. Nevertheless, it is possible to affirm that the positive effect on height was independent of the presence of microorganisms in the compost extract.

Regarding fresh and dry weights, no differences were observed between treatments when fresh and dry weight of shoots and roots were analyzed (data not shown). Mean fresh weights (gr) were in the range of (4.3 ± 0.5) - (7 ± 1) and (5.4 ± 0.6) - (7.8 ± 1) in roots and shoots, respectively. Mean dry weights (gr) were in the range of (0.9 ± 0.2) - (1.5 ± 0.2) and (2.5 ± 0.3 - 3.5 ± 0.6) in roots and shoots, respectively.

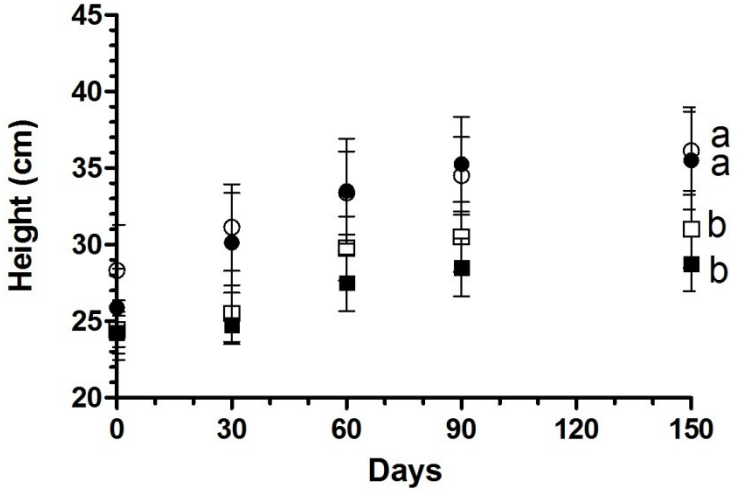


**Figure 2:** *Myrcinathes cisplatensis* height during the experiment. Eleven months old *M. cisplatensis* seedlings were transplanted to 1lt pots filled with autoclaved substrate with (▪, ○, ●) or without (□), autoclaved TC arbuscular mycorrhizal fungi. Three days after the transplant pots were irrigated with 50 mL of a dilution of compost extract previously filtered or not filtered through a nanopore filter (1:500,000 dilution without filtration (●); 1:500,000 dilution filtered (○); AMF only (▪). Plants were grown for 6 months and were irrigated regularly with low P Hoagland solution. Data are expressed as means ± SEM (N = 4). Values with the same lower-case letters in a graph do not differ at $P \leq 0.05$ at 150 days (repeated measure test).

The methodological approach used in this study (the production of soil enriched with AMF indigenous populations and the use of compost extract to promote AMS) could be applicable in agroecological transitions that incorporates trees in the agroecosystem design. Planting young trees that are already in symbiotic association with AMF populations isolated from the field where trees will be transplanted can help the trees to

overcome stress during the transplant (Rivillas; Calle; Angel. 2019). Moreover, it could promote the restoration of soil life since AMF mycelium has the potential to interconnect with the pre-existing CMN and thus help to stabilize AMF mycelium that could become AMS inoculum for future crops (Giovannetti; Avio; Sbrana, 2015). In addition, the promotion of AMS in agroecosystems has also the potential to stimulate carbon sequestration into the soil, thus contributing to the mitigation of climate change (Moriën *et al.*, 2017).

As a possible strategy to develop a sustainable agriculture, plenty of efforts are currently put into the development of biological products that contain isolated species of microorganisms or a mixture of them. Overall, our results demonstrate that another strategy can be undertaken, a strategy that stimulates the growth of the indigenous microorganisms populations, in this case of AMF, and that the symbiosis can be stimulated with extremely diluted compost extracts solutions possibly due to the presence of soluble biomolecules. Moreover, the techniques used in the study (i.e. compost and trap culture of indigenous AMF) are not expensive and could be implemented by native trees plant nurseries and/or family farmers. Particularly, we demonstrated that a 1:500,000 dilution of a compost extract stimulated AMS in *M. cisplatensis* seedlings.

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